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Providing Historical Control Data for Toxicology With the SAS Stored Process Web Application

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ABSTRACT

In standard toxicological tests historical control data are used to assess the validity of the experiments. Different ways of determining control limits derived by historical control data will be presented.

Small applications are build using SAS Stored Processes to gather historical control data in small databases and to calculate control limits.

Using the SAS Stored Process Web Application and the security mechanism of the SAS BI Infrastructure these applications can be delivered to the toxicologists all over the enterprise.

WHAT ARE HISTORICAL CONTROL DATA?

Among the critical stages in the development of a new product preclinical animal studies are often conducted to assess the toxicity of the study drug. With the assumption that the results from animal studies can predict results in humans, preclinical testing for toxicity provides valuable information regarding the safety of the study drug. To accurately and precisely assess the toxicity of the study drug, valid statistical design and analysis are necessarily employed. Standardized testing for toxicity is usually designed based on a fixed protocol.

A plethora of variables (e.g., genetic, environmental, infectious agents) can potentially affect the outcome of studies performed with animals. It is therefore critical to use control animals to minimize the impact of these extraneous variables or to recognize the possible presence of unwanted variables. Multiple types of controls include positive, negative, vehicle, and comparative.

POSITIVE CONTROLS

In positive control groups, changes (usually to a high extent) are expected. Positive controls are used to demonstrate that a response can be detected, there-by providing some quality control on the experimental methods. The effect of the positive control is expected not vary from study to study.

NEGATIVE CONTROLS

INegative controls are groups of subjects that completely feft untreated an unaffected. Negative controls are expected to produce no change from the normal state. The purpose of the negative control is to ensure that an unknown variable is not adversely affecting the animals in the experiment, which might result in a false-positive conclusion.

VEHICLE CONTROLS

A vehicle control is used in studies in which a substance is used as a vehicle for a solution of the experimental compound. In a vehicle control, the supposedly innocuous substance is used alone, administered in the same manner in which it will be used with the experimental compound. When compared with the untreated control, the vehicle control will determine whether the vehicle alone causes any effects. Conceptually the same are sham and placebo controls

COMPARATIVE (STANDARD) CONTROLS

A comparative or standard control is often a positive control with a known treatment that is used for a direct comparison to a different treatment.

AN EXAMPLE: THE IN VIVO MICRONUCLEUS TEST (MNT

The MNT is carried out with the following experimental design:

Group	Treatment	Dose level	24h	48h
1	Vehicle control		+	+
2	Test substance	Low dose	+	-
3	Test substance	Mid dose	+	-
4	Test substance	High dose	+	+
5	Positive control		+	-

The study method has a repeated measures parallel design, with two measurement time points (24h and 48h after administration). At the second time point (48h), only the vehicle control and the high dose group are measured.
Male or female or both sexes are used in a study. Usually five animals per sex are included into a study.
The following study parameters are measured during the study:

Parameter	Description
PCE	Number of polychromatic erythrocytes per 1000 NCEs
NCE	Number of normochromatic erythrocytes
PCE (M)	Promille of micronucleated PCEs calculated from 2000 PCEs
NCE (M)	Promille of micronucleated NCEs calculated from 2000 NCEs

The MNT is carried out routinely as a standard test for genotoxicity. The measurements for the control groups are collected separately for each study and form the historical control data.

BUILDING DATABASES OF HISTORICAL CONTROL DATA

Historical control data are collected for each standard toxicological test. To do this each working group for a specific test has its own Excel-based system to maintain their list of historical control data and prepare yearly reports containing simple descriptive statistics. Inspired by our previous efforts to use SAS Stored Processes to evaluate standard toxicological tests our department got the request from the Genetic Toxicology department to provide an application for the evaluation of historical control data for all test systems in Genetic Toxicology.
Let us see how we did this and stick to the example above, the MNT.

THE DATA STRUCTURE

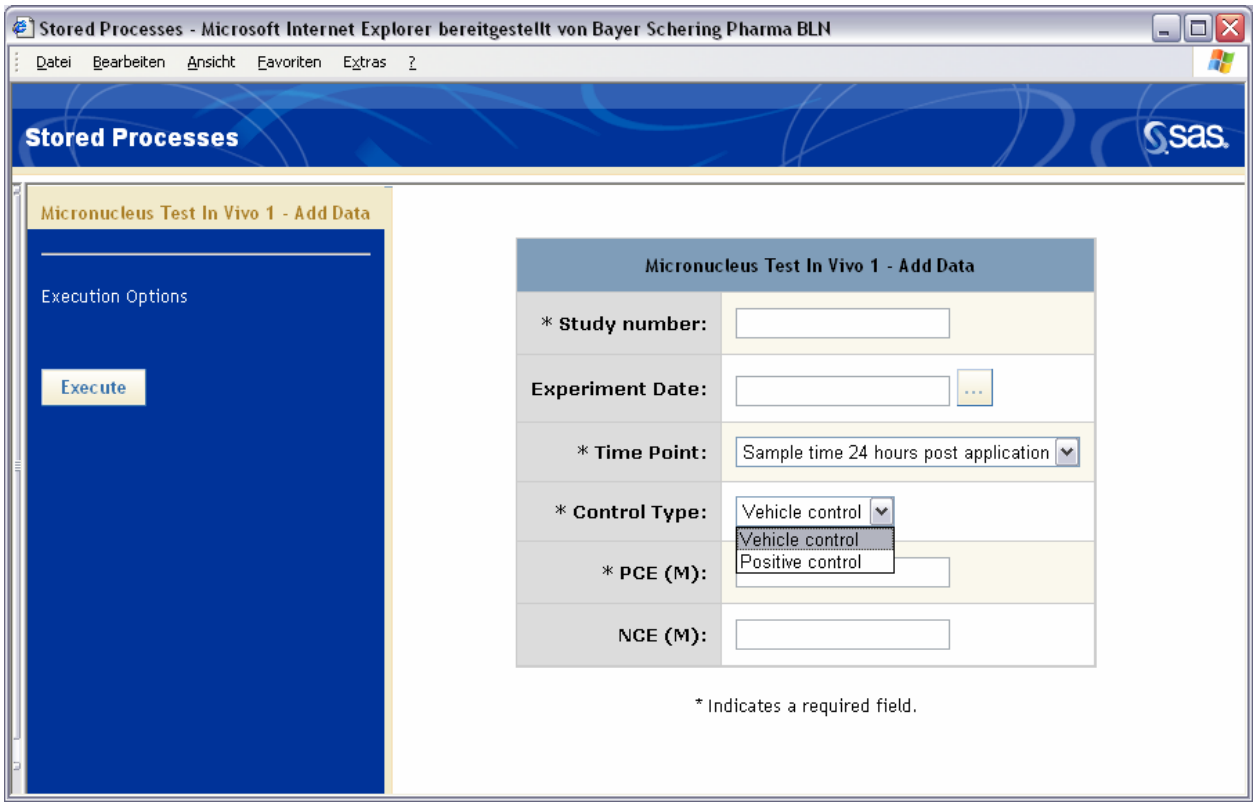
For the MNT for each experiment the means for the parameters PCE (M) and NCE(M) for the negative control group and the mean of parameter PCEM for the positive control group for both time points are collected.
That means for a database we get the following data structure:

```
proc format;
    value TimePointf   1 = "Sample time 24 hours post application"
                       2 = "Sample time 48 hours post application";
    value ControlTypef 1 = "Negative control"
                       2 = "Positive control";
run;
proc sql;
    create table db.HCDMNTOne
        (
            /* Keys */
            StudyNo         char (12) label = 'Study Number',
            ExperimentDate  num       label = 'Experiment Date' format IS8601DA10.,
            /* Groups */
            TimePoint       num       label = 'Sample Time'    format TimePointf.,
            ControlType     num       label = 'Control type'   format ControlTypef.,
            /* Values    */
            PCEM            num       label = 'PCE (M)',
            NCEM            num       label = 'NCE (M)'
        );
quit;
```

StudyNo is a unique identifier for an experiment, the experiment date is needed to do evaluations over a time frame.
TimePoint and ControlType reflect the design of the test; PCEM and NCEM are used to collect the values.

MAINTAINING THE DATABASE

To build up and maintain the database we developed a small application using SAS Stored Processes.
The database is built up from scratch, because there are not so many values. To do this the SAS Stored Process “Add Data – In Vivo Micronucleus Test 1” with the following graphical inbterface is used:



This SAS Stored Process is used to enter the means of the parameter values PCE (M) and NCE (M) for each time point and each type of control group for a study carried out on a certain experiment date.

For maintenance of the database there are SAS Stored Processes “Delete Data – In Vivo Micronucleus Test 1” to delete data of a study and a SAS Stored Process “Report Database – In Vivo Micronucleus Test 1”, which lists the data in the database.

The functionality is implemented by simple proc sql and proc print statements.

REPORT HISTORICAL CONTROL DATA

Current practice for evaluating the historical control data is the calculation of mean, range and standard deviation for each parameter and control group for a given time frame.

For a compact implementation of these reports we use handy combination of proc sql and proc report:

```
proc sql;
    create table Summary as
        select
            ControlType,
            Timepoint,
            count (*)
                as NoOfStudies label = 'Number of studies',
            catx(' '||byte (177)||' ',round(mean(PCE),0.01),round(std(PCE),0.001))
                as MeanStdPCE  label = "&MVPlusMinusSD.",
            catx(' - ',round(min(PCE),0.01),round(max(PCE),0.01))
                as RangePCE    label = "Range",
            catx(' '||byte (177)||' ',round(mean(NCE),0.01),round(std(NCE),0.001))
                as MeanStdNCE  label = "&MVPlusMinusSD.",
            catx(' - ',round(min(NCE),0.01),round(max(NCE),0.01))
                as RangeNCE    label = "Range"
        from db.HCDMNTOne
        group by ControlType, TimePoint;
quit;
```

By combining functions in proc sql for descriptive statistics and character functions it is easy to construct the typical table entries for this kind of reports. (Macro variable MVPlusMinusSD contains the value byte(177)).

```
proc report data = Summary split='*' nofs;
    break after ControlType/dol;
    columns
        ControlType
        TimePoint
        NoOfStudies
       ('Micronucleated cells (‰) scored in 2000 PCE/1000 NCEs per animal'
           ('PCE (M)' MeanStdPCE RangePCE) ('NCE (M)' MeanStdNCE RangeNCE)
        );
    define ControlType/group;
    define TimePoint/order 'Sample Time * (hours post application)';
    define NoOfStudies/display 'Number * of studies';
    define MeanStdPCE/display center;
    define RangePCE/display center;
    define MeanStdNCE/display center;
    define RangeNCE/display center;
    title1 'Compilation of Historical Control Data (Pooled Mean Values) of PCE (M)
            and NCE (M) for Micronucleus Test in Male and Female Mice';
    title2 'One application';
run;
```

As seen in the columns statement we heavily use the ability to stack headers in proc report.
These two statements produce the following report:

Compilation of Historical Control Data (Pooled Mean Values) of PCE (M) and NCE (M) for Micronucleus Test in Male and Female Mice
One application

			Micronucleated cells (‰) scored in 2000 PCE/1000 NCEs per animal			
			PCE (M)		*NCE (M)*	
Control type	*Sample Time (hours post application)*	*Number of studies*	*MV ± SD*	*Range*	*MV ± SD*	*Range*
Negative control	24	42	0.89 ± 0.119	0.7 - 1.3	0.94 ± 0.227	0.4 - 1.6
	48	42	0.85 ± 0.152	0.4 - 1.2	0.8 ± 0.18	0.2 - 1.2
Positive control	24	42	11.75 ± 3.048	5.95 - 20.2		

WAYS TO DETERMINE CONTROL LIMITS OF HISTORICAL CONTROL DATA

With the above steps we have captured the as is solution and converted it to a solution, which is centrally deployed and enterprise wide available as well as extensible by all means of SAS.
As the historical data are collected to implement some kind of quality control it is desirable to have an estimation of what is acceptable in variation. These acceptability ranges are called control limits. As we have in our department a long history of using SAS to evaluate toxicological studies, we can use a whole bunch of methods to extend the the above solution with more or less sophisticated methods to get control limits.

REFERENCE RANGES

A reference range for a particular test or measurement, is usually defined as the values that 95% (or 2 standard deviations) of the population fall into. It relies on the fact that for many biological phenomena, there is a normal distribution of values. This is often the first step used in a laboratory environment.

DISTRIBUTION-FREE TOLERANCE LIMITS

As the assumption of a normal distribution of values in toxicological experiments often is not adequate we have a SAS macro in use that provides distribution-free tolerance intervals. This also can be used to easily extend the application.

CONTROL CHARTS

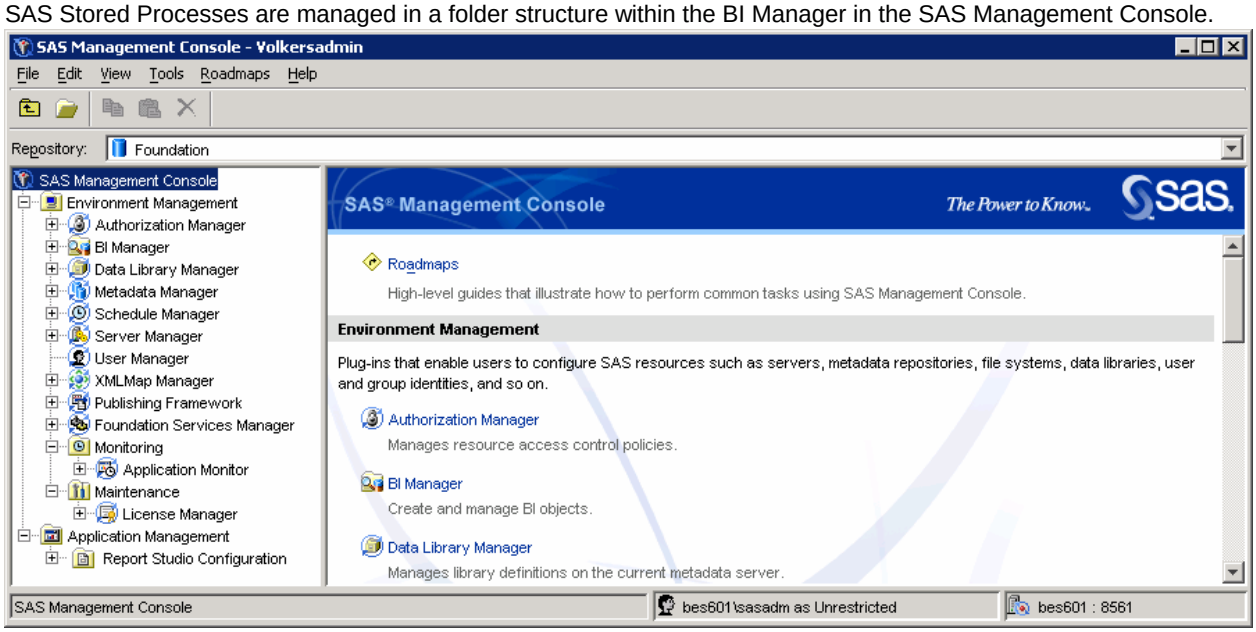
A third way to determine control limits practiced in our department (see PhUSE 2007) are control charts. here, control limits are horizontal lines drawn on an statistical process control chart, usually at a distance of ±3 standard deviations of the plotted statistic from the statistic's mean. For normally distributed statistics, the area bracketed by the control limits will on average contain 99.73% of all the plot points on the chart, as long as the process is and remains in statistical control.

BUILDING UP APPLICATIONS FROM SAS STORED PROCESSES

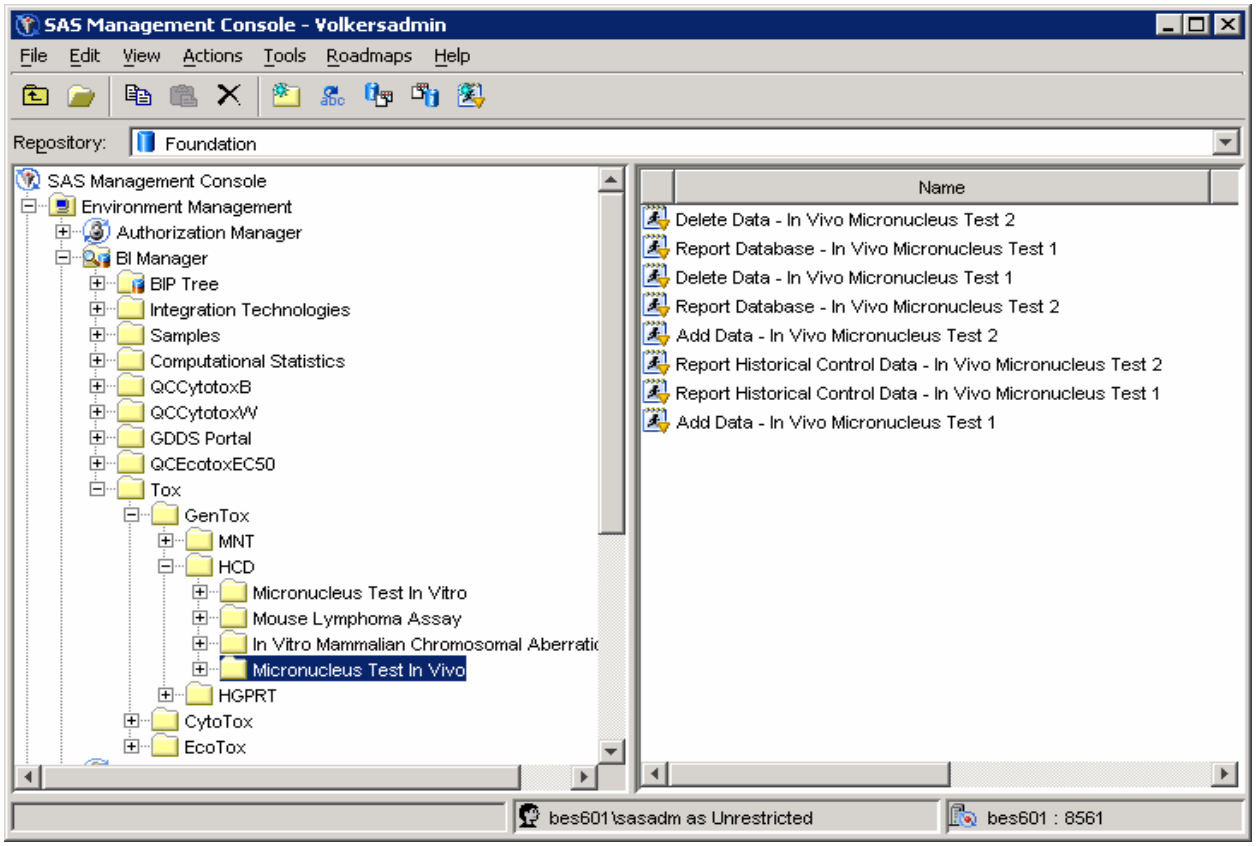
So far we have described how the different feature can be implemented as SAS Stored Processes. But how do we organize all that simple function to application that can be presented to different user groups.

SAS MANAGEMENT CONSOLE´S BI MANAGER TREE

SAS Stored Processes are managed in a folder structure within the BI Manager in the SAS Management Console.



For the toxicology departments we built up a subtree “Tox” in the BI Manager containing the metadata of all SAS Stored Processes.

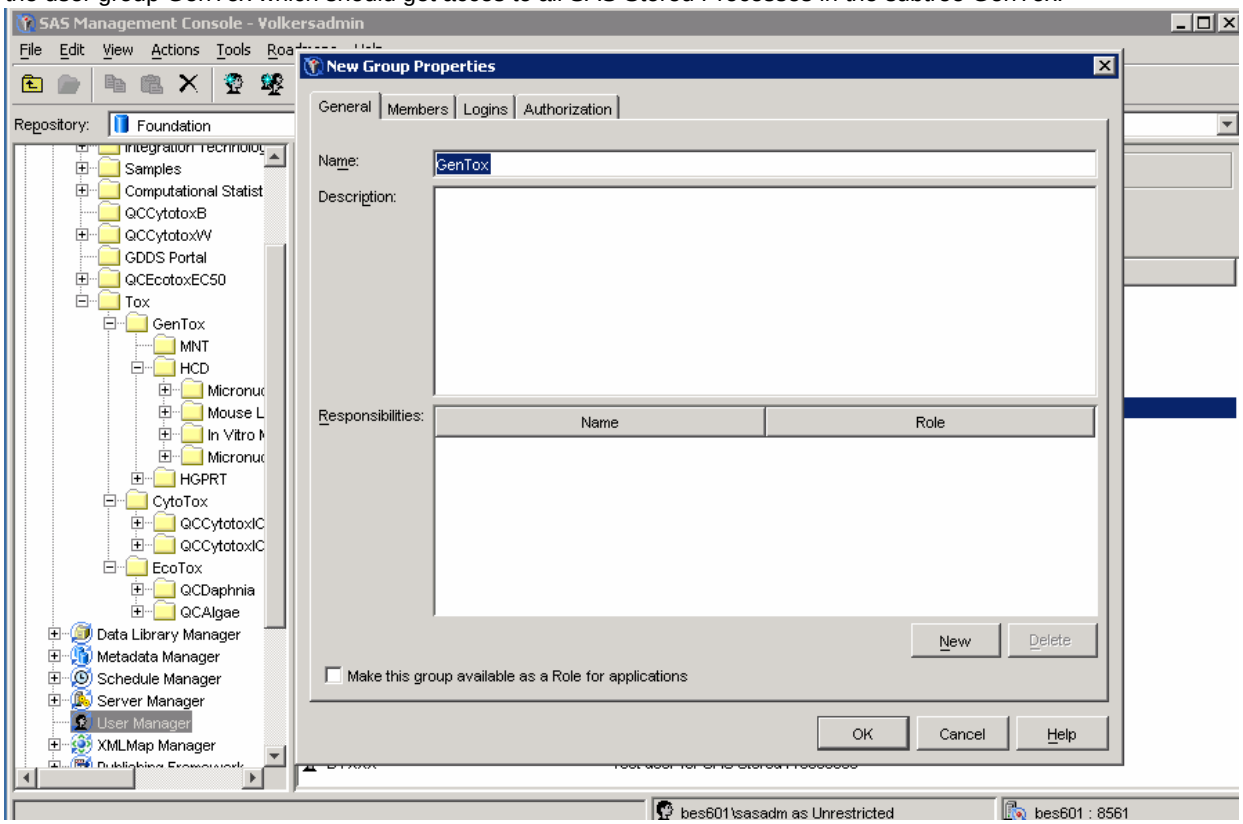


The screen shot shows the SAS Stored Processes that are used to maintain the two databases of historical control data for the Micronucleus Test In Vivo. SAS Stored Processes for the other test systems are grouped in further folders.

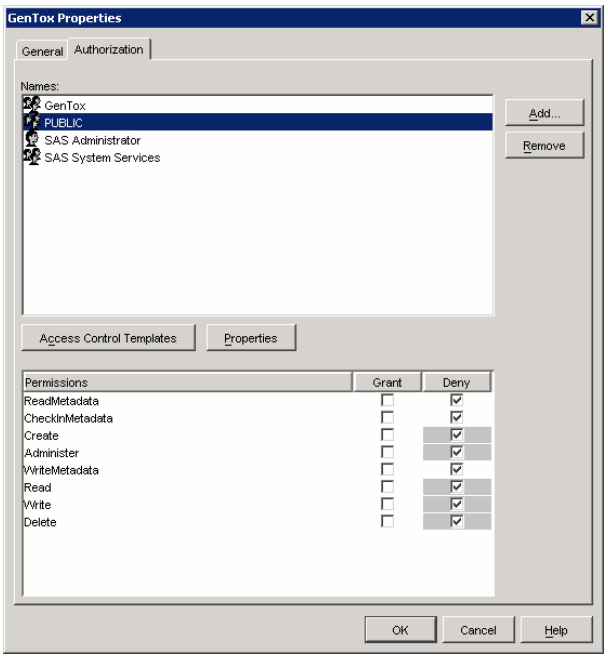
DEFININIG ACCESS RIGHTS TO STORED PROCESSES

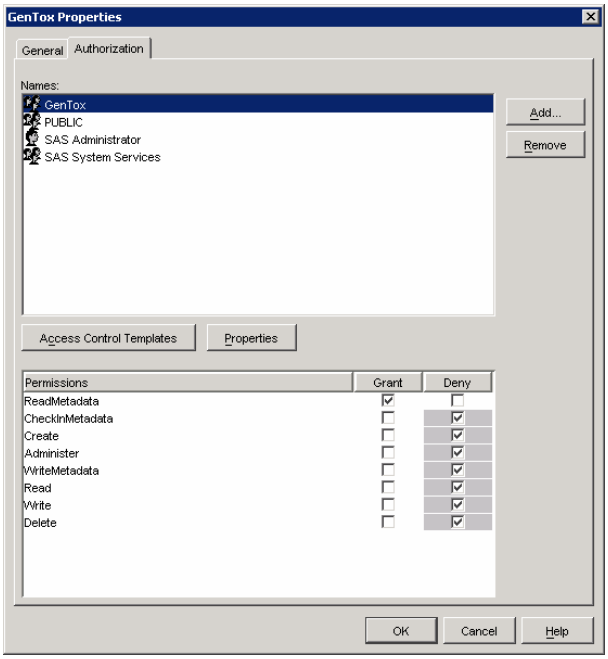
This grouping can be used to conviently grant access right to users and user groups.

To define a user group the User Manager plug-in of the SAS Management Console is used. as an example we create the user group GenTox which should get acces to all SAS Stored Processes in the subtree GenTox.



To provide the users group GenTox an authorized access to the subtree GenTox we have to edit the properties of GenTox with BI Manager. Under the authorization tab we find a list of the user groups and the permissions for the currently selected group

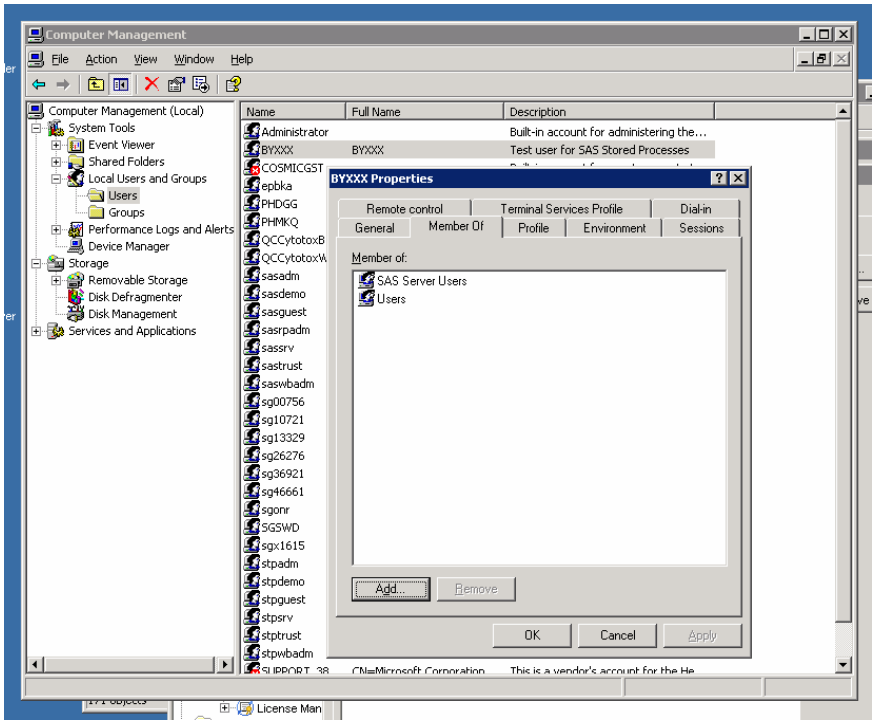




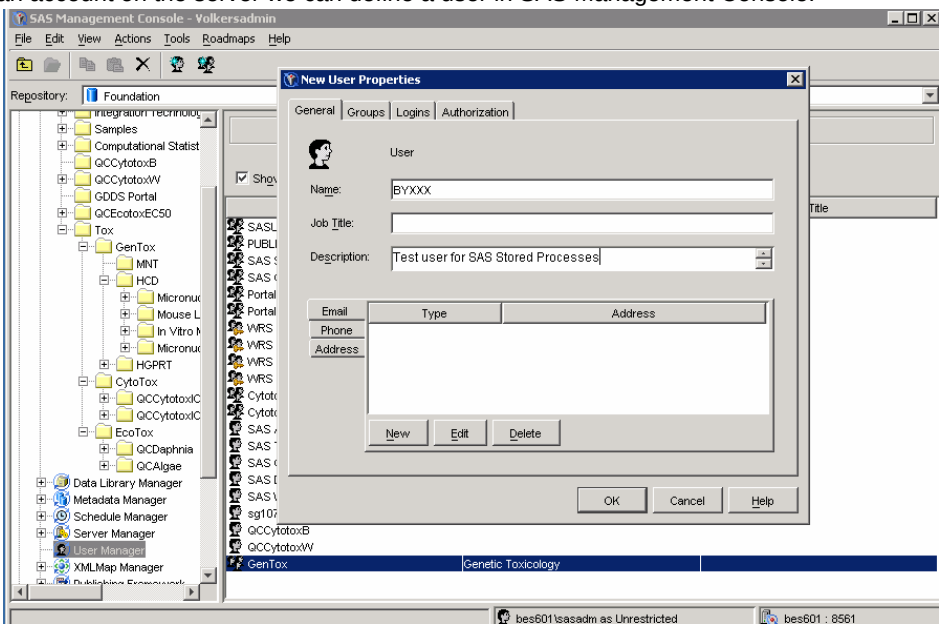
As a first step we have to deny access to the PUBLIC group, which is the group that has unauthorized access. as a second step, we have grant the right “ReadMetadata” to the GenTox group allowing to execute the SAS Stored Processes contained in this subtree.

GIVING USERS ACCESS TO SAS STORED PROCESSES

For a user to get access to SAS Stored Processes he needs to get authorized access to the server the SAS Metadata is running on as well as to the Metadata of the SAS Stored Processes.

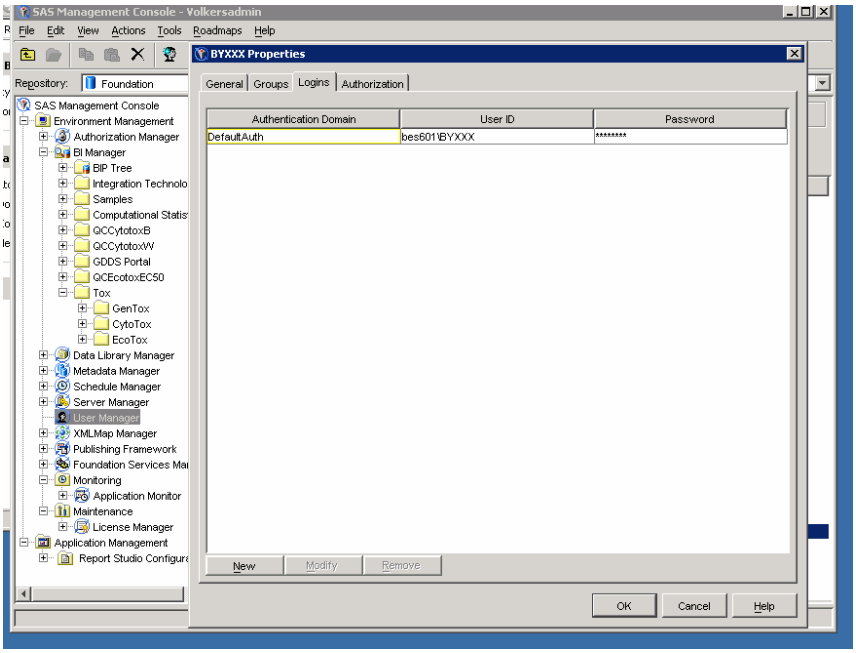


On the server the user has to be member of the SAS Servers Users, a group that is created during SAS installation. Now we have an account on the server we can define a user in SAS Management Console.

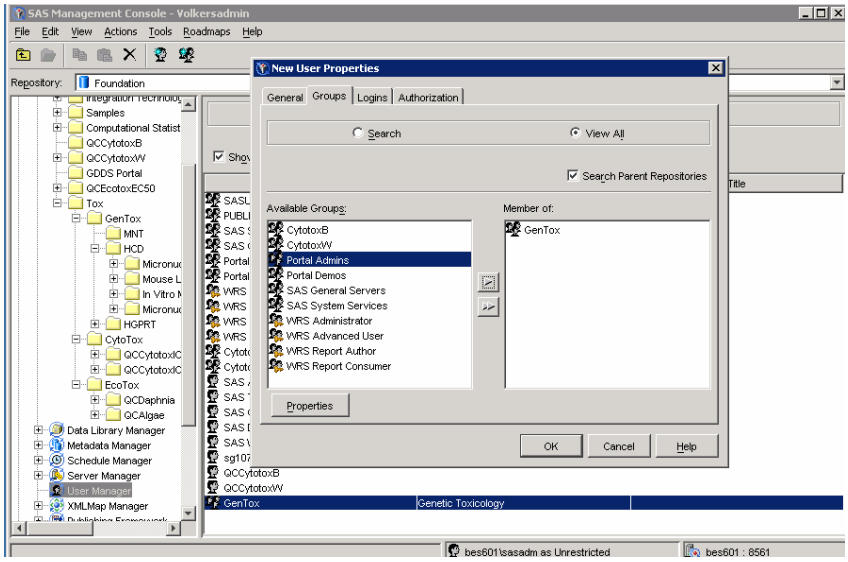


This is done by going to the User Manager going to Actions/New/User. Here we define the user entitiy, which gets access to the SAS Stored Processes. This done under the General tab.

The next step is to enter the user identity of this user on the server. This is done under the tab Logins. In a standard installation the Authentication domain will be DefaultAuth, User ID and Password have to be the ones defined on the server as they are used to access it.



To allow access o the SAS Stored Processes for Genetic Toxicology under the tab Groups we assign him to group GenTox.



THE SAS STORED PROCESS WEB APPLICATION

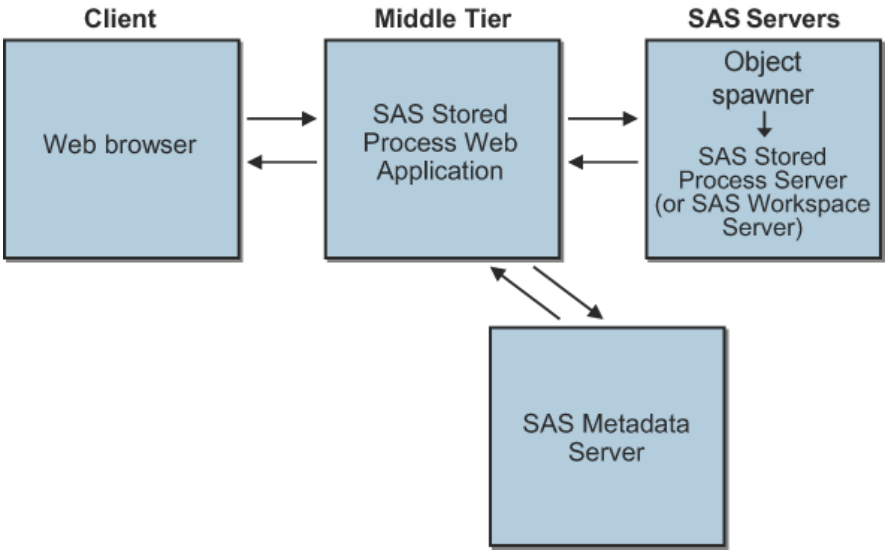
Now the SAS Stored Process Web Application comes in to make sense of it all.

The SAS Stored Process Web Application is a Java Web application that can execute stored processes and return results to a Web browser.

Here's how the SAS Stored Process Web Application processes a request:

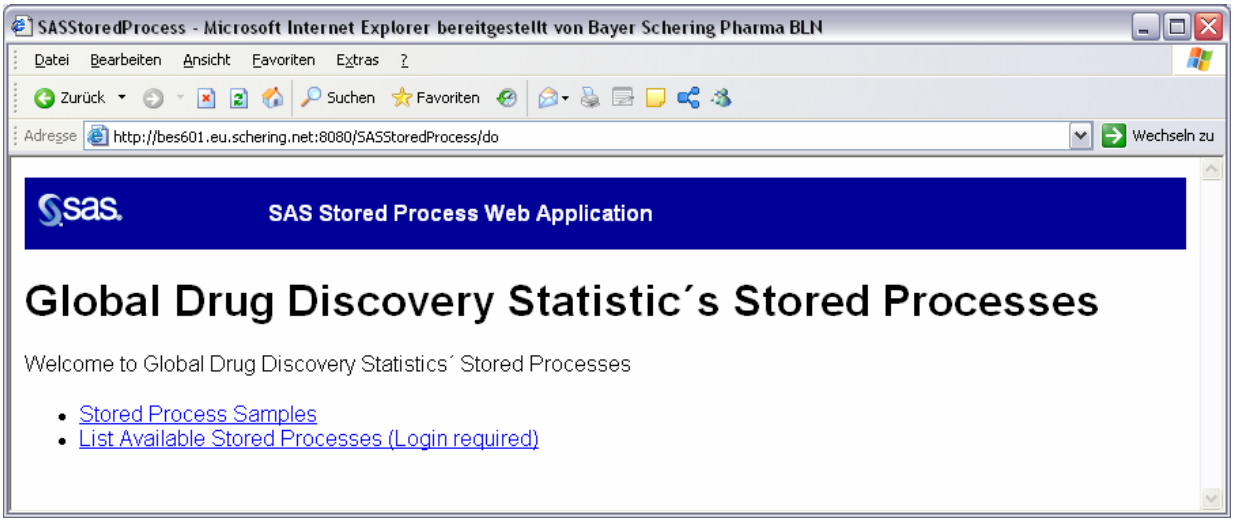
Users enter information in the HTML form of the SAS Stored Process by using their Web browser and then submitting it. The information is passed to the Web server, which invokes the first component, the SAS Stored Process Web Application. The SAS Stored Process Web Application accepts data from the Web server and contacts the SAS Metadata Server for user authentication and retrieval of stored process information. The stored process data is then sent by the SAS Stored Process Web Application to a stored process server through the object spawner. The stored process server invokes the SAS program assigned to the SAS Stored Process, that processes the information. The results of the SAS program are sent back through the Web application and Web server to the Web browser of the user.

The following diagram illustrates this process:



THE WELCOME PAGE

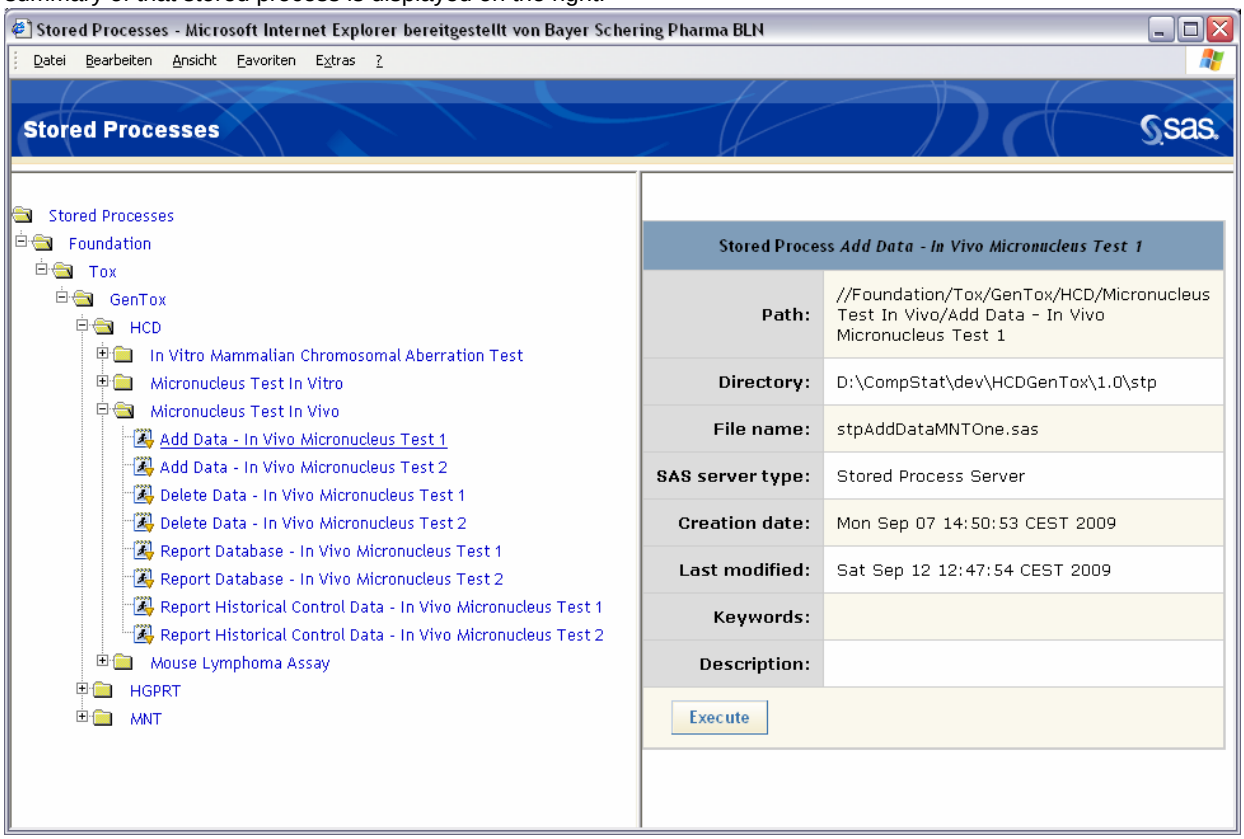
The SAS Stored Process Web Application is delivered with a Welcome Page, which can be accommodated to your needs or even be omitted. This Welcome Page will be accessed via a web browser and in our case it has the following look:



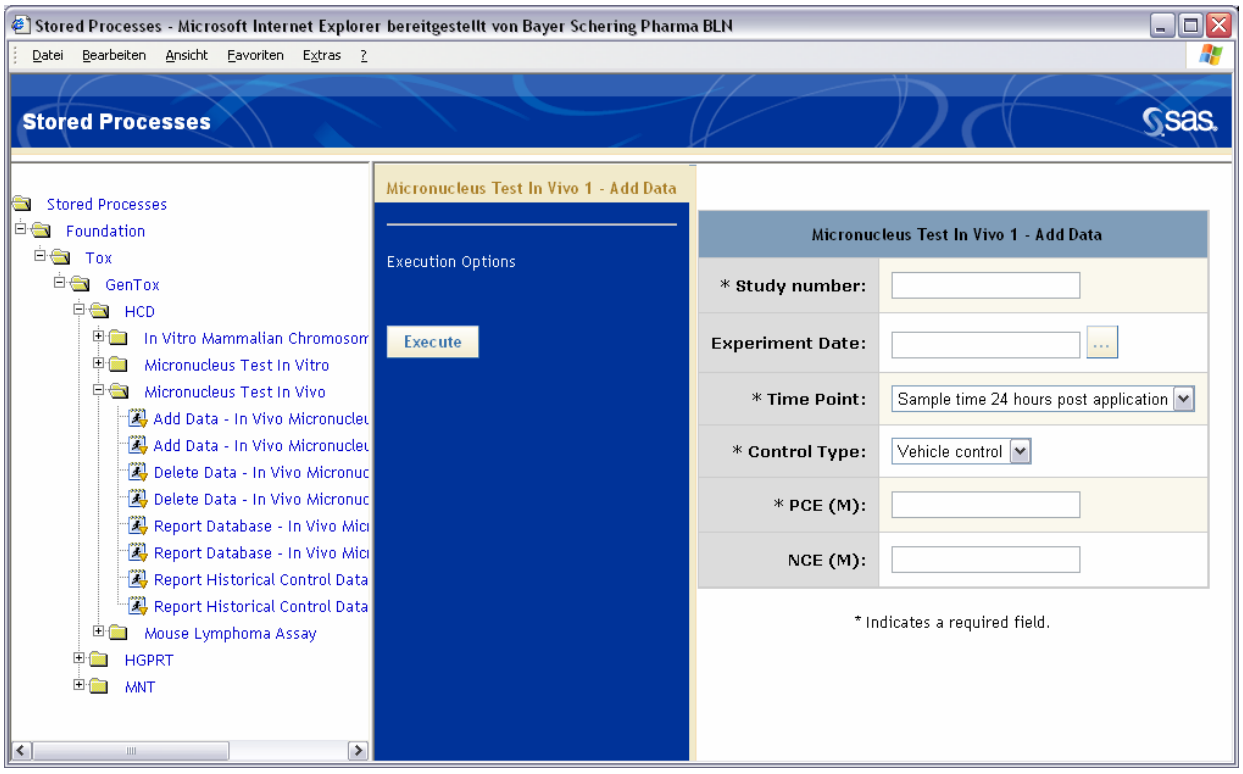
The link Stored Process Samples points to the examples provided by SAS, the other link points to the Login to get authorized access to the provided SAS Stored Processes:

THE LIST OF AVAILABLE SAS STORED PROCESES

After authentication a page is displayed a page that is split into sections. On the left is a tree view of metadata repositories, folders, and stored processes. If you drill down in the tree view and select a stored process, then a summary of that stored process is displayed on the right:



The content presented here exactly represents what was authorized in the SAS Metadata.
Clicking Execute at the bottom of the summary runs the stored process.



CONCLUSION

The GDDS Statistics Portal as described in previous papers converted to a portal idea implemented by the SAS Stored Process Web Application. Using these tools delivered by SAS BI Server it is possible to implement enterpris-wide applications tailored to special needs and user groups without any special interface programming.

REFERENCES

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References go at the end of your paper.

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